Effect of magnesium deficiency on thiamin metabolism*)

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Zusammenfassung

Es wurde der Thiamin-Stoffwechsel bei Ratten im Mg-Mangel untersucht mit folgenden Ergebnissen:

1. Bei Mg-verarmten Ratten war der Gehalt an Thiamin in Leber, Herz und Nieren gegenüber den Kontrollen erniedrigt. Bei Mangel-Ratten wurde keine Abnahme des Thiamins im Zentralnervensystem gefunden.
2. Die Aktivitäten Thiamin-abhängiger Enzyme der Leber wie Pyruvatdehydrogenase und Transketolase waren bei Mangeltieren vermindert.
3. In subzellularen Leberfraktionen von Mangeltieren war der Thiamin-Gehalt besonders eindrucksvoll in der Mitochondrienfraktion erniedrigt.
4. Nach Gabe von ^{14}C-Thiamin fand sich bei Mangelratten eine Abnahme des Thiamin-Gehalts im 24-h-Urin, und im Vergleich zu Kontrollen stieg die Radioaktivität in Blut, Leber, Nieren, Herz und Nervus ischiadicus an.

Diese Ergebnisse zeigen, daß Mg eine Rolle bei der Fixierung von Thiamin in Geweben spielt, vor allem im Thiamin-abhängigen Enzymsystem der Leber.

Summary

Experiments for invensting thiamin metabolism in magnesium deficient rats were carried out, and the following results were obtained.

1. Thiamin concentration in the liver, heart and kidneys of magnesium deficient rats was lower than in the magnesium sufficient rats. A decrease in thiamin was not detected in the central nervous system of magnesium deficient rats.
2. Activities of thiamin dependent enzymes, i. e. pyruvate dehydrogenase and transketolase, in liver had decreased in magnesium deficient rats.
3. In subcellular fractions of the liver of magnesium deficient rats, thiamin content was most markedly decreased in the mitochondrial fraction.
4. When ^{14}C-thiamin was administered to magnesium deficient rats, thiamin content in 24-hour urine decreased, and the radioactivity in the blood, liver, kidneys, heart and sciatic nerve increased as compared to magnesium sufficient rats.

These results indicated that magnesium plays a role in binding thiamin in tissues, especially in the thiamin dependent enzyme system in liver.

Résumé

Nous avons effectué des expériences pour étudier le métabolisme de la thiamine chez des rats avec déficit magnésique et nous avons obtenu les résultats suivants:

1. La concentration de thiamine dans le foie, le coeur et les reins des rats avec déficit magnésique a été plus faible que chez les rats avec une ration suffisante en Mg. Une réduction de la thiamine n'a pas été décelée dans le système nerveux central des rats avec déficit magnésique.
2. Les activités des enzymes dépendant de la thiamine, entre autres de la pyruvate-déhydrogénase et de la transkétolase, dans le foie ont ète réduites chez les rats avec déficit magnésique.
3. Dans les fractions subcellulaires du foie des rats avec déficit magnésique, la teneur en thiamine a été le plus nettement réduite dans la fraction mitochondriale chez les rats avec déficit magnésique.
4. Quand de la thiamine-^{14}C a été administrée à des rats avec déficit magnésique, la teneur en thiamine dans l'urine des 24 h a été réduite et la radioactivité dans le sang, le foie, les reins, le coeur et le nerf sciatique s'est accrue, par comparaison avec des rats avec un régime suffisant en Mg.

Ces résultats indiquent que le Mg joue un rôle dans la fixation de la thiamine dans les tissus, et en particulier dans le système enzymatique, dépendant de la thiamine, dans le foie.

Introduction

Many reports confirmed that dietary thiamin levels have a considerable influence on magnesium deficiency, characterized by growth response [9], peripheral erythema and blood serotonin levels [7], utilization of thiamin [8], transketolase activity [10], mineral concentration in various tissues [5] and lipid metabolism [6]. The present study is intend to clarify the difference of thiamin metabolism between magnesium sufficient and magnesium deficient animals.

Materials and methods

Animals and Diets

Experiment No. 1: Male rats of Wistar strain, weighing 80—100 grams, were separated into two groups. To one group a magnesium sufficient diet (observed magnesium concentration: 68.8 mg/100 g of diet) was given and to another group a magnesium deficient diet (observed magnesium concentration: 5.8 mg/100 g diet) was given. The rats were housed individually in stainless steel cages. After 30 days of these dietary regimens, the rats were killed and heart, liver, kidney, brain, spinal cord and sciatic nerve were removed.

Experiment No. 2: The above dietary regimens were carried out for 30 days to other rat groups and then 1 mg of ^{14}C-thiamin (1.4 mCi/mmole) was injected intraperitoneally after which a 24 hour urine specimen was collected. Then rats were killed.

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Experiment No. 3: Rats were separated into four groups and four synthetic diets (thiamin deficient-magnesium deficient, thiamin deficient-magnesium sufficient, thiamin sufficient-magnesium deficient and thiamin sufficient-magnesium sufficient) were administered. Food consumption of thiamin sufficient groups was measured daily to determine the thiamin intake. To rats fed a thiamin deficient diet, the same amount of thiamin was given by intraperitoneal injection. These routines were carried out for 30 days.

Analytical procedures

Thiamin concentration in various tissues were determined by the thiochrome method of *Fujiwara* and *Matsui* [2]. Pyruvate dehydrogenase (EC 1.2.4.1) and transketolase (EC 2.7.1.1) activities in liver were assayed as described by *Itokawa* [4]. Each enzyme assay was carried out an incubation of liver homogenates with ribose-5-phosphate as a substrate in the both the presence and the absence of excess thiamin pyrophosphate (TPP). After the incubation, the production of sedoheptulose-7-phosphate is measured. The percentage stimulation of transketolase activity produced by adding TPP represents the amount of the apoenzyme not saturated with thiamin (TPP effect) [1]. For separation of liver subfractions, a portion of liver was homogenized in a 0.25 M sucrose solution containing Tris buffer (0.5 mM) at a pH of 7.4. Nuclei, mitochondria, microsome and supernatant fractions were separated centrifugally by the method of *Hogeboom* [3]. Radioactivity was determined by adding 0.2 ml of urine, blood, or tissue homogenate to 20 ml of counting solution which consisted of toluene, triton X-100, PPO and dimethyl POPOP using a liquid scintillation counter. Sample quenching was corrected by the channel ration technique.

Results

Experiment No. 1

Thiamin concentration in various tissues are shown in Figure 1. Thiamin concentration in the heart, liver, kidney and sciatic nerve in the magnesium deficient rats was lower than in magnesium sufficient rats. On the other hand, no significant difference was observed between magnesium deficient and sufficient rats in the central nervous system.

Figure 2 shows the activities and TPP effect in pyruvate dehydrogenase and transketolase in the

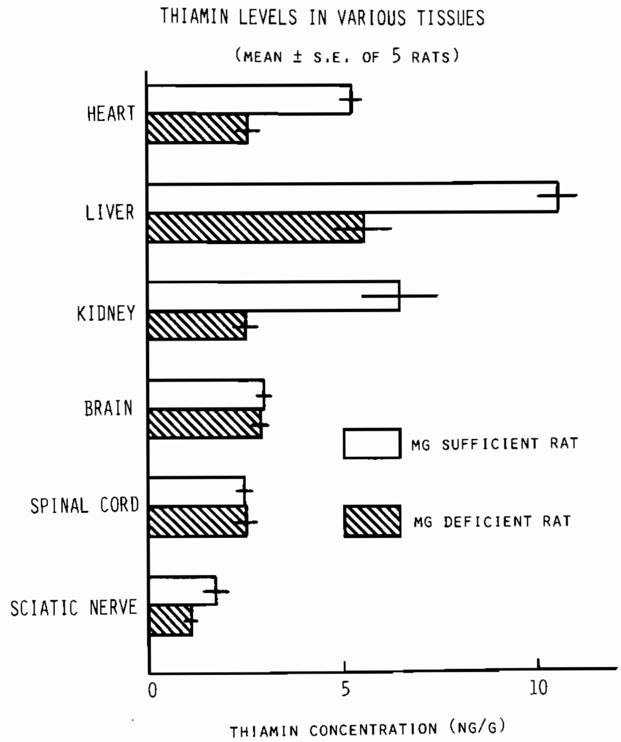

Fig. 1

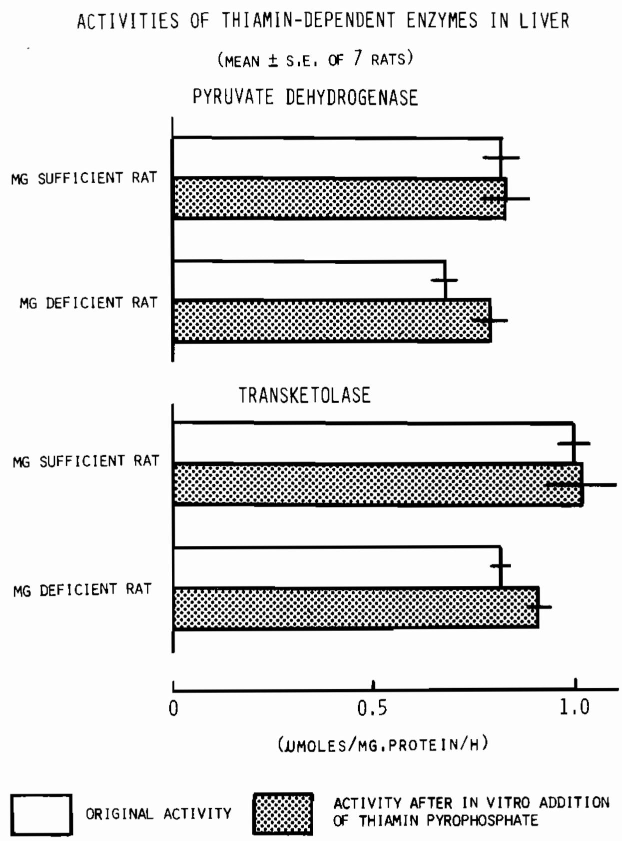

Fig. 2

liver. The activities of both enzymes decreased in magnesium deficient rats. TPP effect in both enzymes increased significantly in the liver of magnesium deficient rats.

Thiamin concentration in liver cell subfractions is shown in Figure 3. The thiamin level in the mitochondrial fraction of the liver was decreased most significantly in magnesium deficient rats as compared to the magnesium sufficient ones.

Experiment No. 2

As represented in Figure 4, radioactivity in the 24-hour urine specimen was higher in magnesium sufficient rats than in magnesium deficient rats. In contrast, ^{14}C-thiamin in the blood, liver and kidney was higher in magnesium deficient rats than in magnesium sufficient rats. However, there was no significant difference in ^{14}C-thiamin levels of brain between magnesium sufficient and deficient rats.

DISTRIBUTION OF THIAMIN IN LIVER CELL SUBFRACTIONS

(MEAN ± S.E. OF 4 RATS)

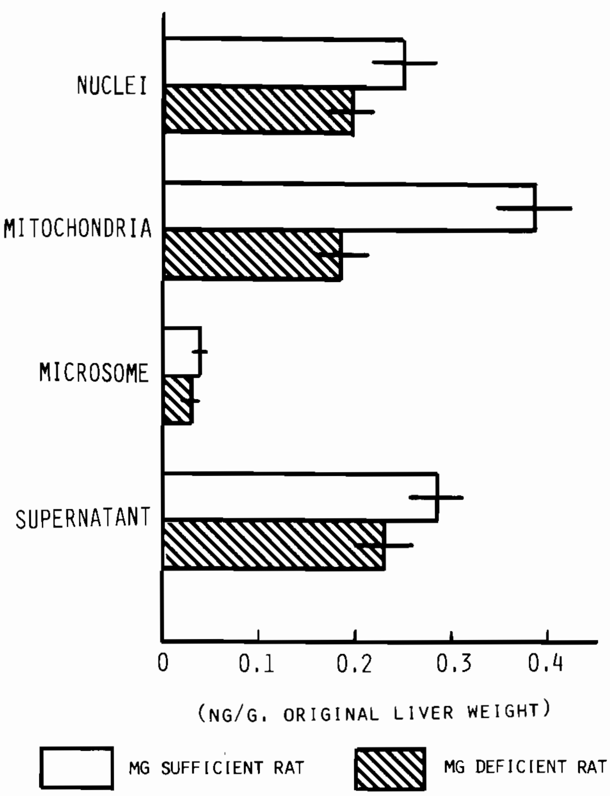

Fig. 3

RADIOACTIVE THIAMIN IN URINE, BLOOD AND TISSUES 24-HR AFTER INJECTION OF ^{14}C-THIAMIN (1 mg) TO RAT

(Mean±S.E. of 4 Rats)

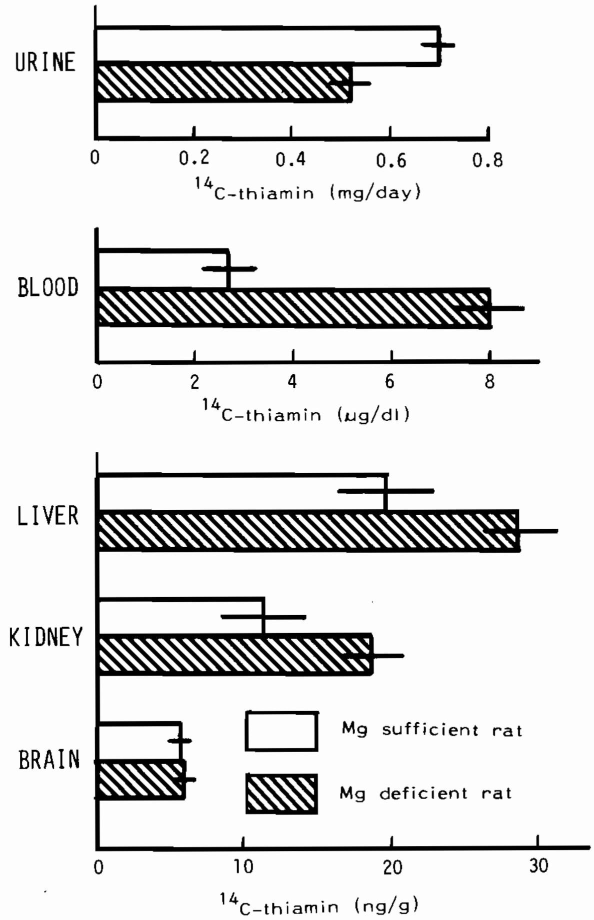

Fig. 4

Experiment No. 3

As indicated in Figure 5, with both oral and parenteral administration of thiamin, thiamin levels in the heart, liver and kidney of magnesium deficient rats decreased significantly as compared to the magnesium sufficient rats. No significant difference was seen among the groups as far as thiamin levels in the brain.

Discussion

The following findings (1)—(3) in the present study, confirm that magnesium deficiency causes a thiamin deficiency. (1) Total thiamin content in the heart, liver, kidneys, and sciatic nerve of magnesium deficient rats was lower than in that of magnesium sufficient rats. (2) Activities of thiamin-dependent enzymes in liver decreased and TPP-effect increased in magnesium deficient rats.

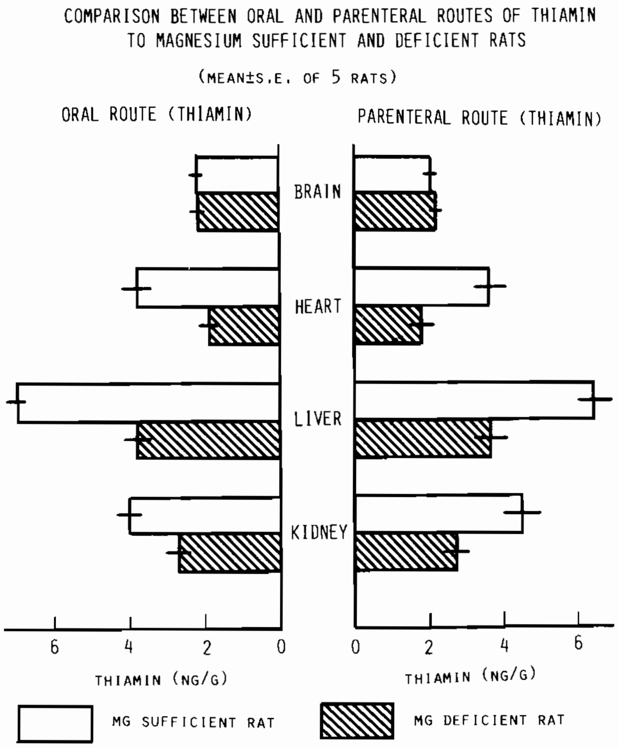

Fig. 5

(3) Radioactivity in the 24-hour urine specimen after loading of ^{14}C-thiamin was lower in the magnesium deficient rats as compared to the magnesium sufficient rats. In contrast, radioactivity in the blood and tissues was higher in the magnesium deficient rats than in the magnesium sufficient rats.

Regarding magnesium deficient rats, there was no signficant difference of thiamin levels in the tissues of orally thiamin administered and parenterally administered rats. This fact contradicts the hypothesis that magnesium deficiency may inhibit intestinal absorption of thiamin. It is assumed that magnesium plays a role in the maintenance of thiamin in cells, presumably binding thiamin to protein in tissues.

Among various subcellular fractions in the liver, thiamin content in the mitochondrial fraction was decreased most markedly in magnesium deficiency. It is feasible that magnesium deficiency primarily inhibits mitochondrial thiamin related enzymes. Further studies should be done to clarify the mechanisms.

Acknowledgements

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